Herpes simplex virus type 1-encoded glycoprotein C contributes to direct coagulation Factor X–virus binding

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The HSV1 (herpes simplex virus type 1) surface has been shown recently to initiate blood coagulation by FVIIa (activated Factor VII)-dependent proteolytic activation of FX (Factor X). At least two types of direct FX–HSV1 interactions were suggested by observing that host cell-encoded tissue factor and virus-encoded gC (glycoprotein C) independently enhance FVIIa function on the virus. Using differential sedimentation to separate bound from free ^{125}I-ligand, we report in the present study that, in the presence of Ca^{2+}, FX binds directly to purified wild-type HSV1 with an apparent dissociation constant (K_d) of 1.5 ± 0.4 μM and 206 ± 24 sites per virus at saturation. The number of FX-binding sites on gC-deficient virus was reduced to 43 ± 5, and the remaining binding had a lower K_d (0.7 ± 0.2 μM), demonstrating an involvement of gC. Engineering gC back into the deficient strain or addition of a truncated soluble recombinant form of gC (sgC), increased the K_d and the number of binding sites. Consistent with a gC/FX stoichiometry of approximately 1:1, 121 ± 6 ^{125}I-sgC molecules were found to bind per wild-type HSV1. In the absence of Ca^{2+}, the number of FX-binding sites on the wild-type virus was similar to the gC-deficient strain in the presence of Ca^{2+}. Furthermore, in the absence of Ca^{2+}, direct sgC binding to HSV1 was insignificant, although sgC was observed to inhibit the FX–virus association, suggesting a Ca^{2+}-independent solution-phase FX–sgC interaction. Cumulatively, these data demonstrate that gC constitutes one type of direct FX–HSV1 interaction, possibly providing a molecular basis for clinical correlations between recurrent infection and vascular pathology.

Key words: atherosclerosis, coagulation, Factor X, herpes simplex virus, Herpesvirus, thrombosis.

INTRODUCTION

HSV1 (herpes simplex virus type 1) is a highly prevalent human pathogen with the ability to injure the vascular endothelium through continuous lifelong subclinical infections [1]. Endothelial damage is a causative factor in the development of atherosclerosis, and, consequently, HSV1 has been suggested to play a role in the development of vascular disease [2–4]. Supporting evidence includes the clinical correlation of fibrin deposits within the microvasculature [5–7], detection of HSV1 genomic material associated with human atherosclerotic plaques [8–10] and animal models using herpes-type viruses [11–14]. While not a predictor of the onset of coronary heart disease, individuals infected with HSV1 have correlated further to an increased risk of death following myocardial infarction [15].

On a cellular level, the vascular pathology associated with HSV1 has been attributed to inducing a pro-coagulant cell phenotype leading to the generation of thrombin [3,16–19], the final protease produced in the coagulation pathway. Normally limited to the site of vascular damage, thrombin generation is initiated when localized aPL (anionic phospholipid) and TF (tissue factor) become exposed and available to circulating clotting factors [20]. TF binds FVIIa (activated Factor VII), forming a cofactor–enzyme complex on aPL that proteolytically activates FX (Factor X) to FXa (activated Factor X). Obligate interactions between FX and FVIIa with aPL are known to require Ca^{2+}. Once produced, FXa is the proteolytic constituent of the prothrombinase complex, which is essential for physiological thrombin production [21].

Previously, we reported that the HSV1 surface has host-derived aPL and TF [22,23]. These enable FX activation independent of the usual requirement for pro-coagulant cells [22], which is likely to be the initiating event in virus-mediated endothelial damage. Using HSV1 mutants, a parallel TF-independent mechanism was also identified involving virus-encoded gC (glycoprotein C) [23]. This second mechanism is an example of molecular mimicry [24], where HSV1 gC has evolved TF-like cofactor function to enhance FVIIa-dependent FX activation [23]. Further evidence from our laboratory showed that sgC (truncated soluble recombinant gC), lacking the transmembrane domain, increased FXa generation in the presence of FVIIa. Interestingly, sgC activity was increased by three orders of magnitude when purified virus was added, suggesting another contributing species on the virus. These enzymatic studies imply a direct interaction between FX and HSV1, viral gC or sgC. In the present work, we investigated the binding of radiolabelled FX to the purified virus and the contributions of gC and Ca^{2+} utilizing gC-deficient HSV1 and sgC. As hypothesized, we report in the present study that FX binds to HSV1 by gC-dependent and -independent mechanisms.

MATERIALS AND METHODS

Virus preparation

African green monkey kidney cells (Vero CCL-81; A.T.C.C.) were grown on Cytodex 2 microcarrier beads (GE Healthcare) in Medium 199 supplemented with 5% (v/v) foetal calf serum

Abbreviations used: aPL, anionic phospholipid; CMV, cytomegalovirus; FVIIa, activated Factor VII; FX, Factor X; FXa, activated FX; gC, glycoprotein C; HBS, Hepes-buffered saline; HSV1, herpes simplex virus type 1; mAb, monoclonal antibody; NS, wild-type HSV1; ns-1, gC-deficient HSV1; PEG, poly(ethylene glycol) 8000; rns, gC-restored HSV1; sgC, truncated soluble recombinant gC; TF, tissue factor; vp, virus particle.

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and 2 μg/ml gentamycin (Gibco). Once cells reached 85 % confluence, they were infected with individual HSV1 strains, and mature virus was harvested from inoculated cell supernatant as described previously [23]. The HSV1 strains used in the present study include: NS, a wild-type low-passage clinical isolate that contains full-length gC [25]; ns-1, a naturally occurring gC-deficient mutant that does not contain gC on its surface [26]; and rns, an engineered form of ns-1 in which gC has been restored and therefore available on the virus surface [27]. Purified virus was stored at -80 °C, and freshly thawed preparations were used in each experiment. Electron microscopy was used to quantify and evaluate the purity of virus preparations as described previously [22] with less than 10 % of each preparation being attributed to non-viral debris. These non-viral particles have been shown by immunogold electron microscopy to be negative for TF and aPL [22] and therefore do not contribute to the pro-coagulant effects reported for purified HSV1. The individual virus strains used had insignificant differences in diameter, as measured using electron microscopy, allowing direct comparisons of the number of ligand molecules bound per vp (virus particle).

Protein preparation

Human coagulation protein FX was obtained commercially (Haematologic Technologies) and radioiodinated using $Na^{125}I$ (GE Healthcare) and Iodogen (Pierce) as detailed in the manufacturer's procedure. ^{125}I-FX preparations were typically 140 000 c.p.m./μg and retained more than 80 % of the enzymatic activity of native FX as determined using a one-stage clotting assay using Innovin (Dade) to initiate coagulation in FX-deficient plasma (Biopool). sgC with a pentahistidine tag was produced in a baculovirus expression system as described previously [28] (kindly provided by Dr Gary Cohen and Dr Roselyn Eisenberg, University of Pennsylvania). sgC preparations were radioiodinated using the same procedure as for FX. The functional activities of ^{125}I-sgC and non-labelled sgC were determined by a FXa-dependent chromogenic assay as described previously [23], and it was demonstrated that iodination had negligible effects on sgC coagulation function. All protein concentrations were determined using the BCA (bicinchoninic acid) method (Pierce) and corroborated by spectrophotometry.

FX and sgC binding to virus

Various concentrations of ^{125}I-ligand were incubated for 35 min with 5.0×10^8 vps in HBS (Hepes-buffered saline) with 0.1 % (v/v) PEG [poly(ethylene glycol) 8000], 1.8 mM Ca^{2+} and 1 mM benzamidine (HBS/PEG/Ca^{2+}/benzamidine) at 21 °C in a 35 μl total volume. Following equilibrium, 10 μl of the ^{125}I-ligand mixture was layered on to a 200 μl cushion of HBS/PEG/Ca^{2+}/benzamidine containing 10 % sucrose in a 400 μl, 25 mm $\times$ 4 mm, microcentrifuge tube. Following centrifugation at 13 000 ***g*** for 10 min, the supernatant was aspirated in steps to avoid mixing the unbound ligand which localized to the top layer. Once the supernatant was removed, the end of the tube was excised, and the amount of radioactivity associated with the virus pellet was determined. Specific ligand binding to virus was defined as the amount of ^{125}I-ligand displaced by a 40-fold excess of unlabelled ligand or as the amount of ^{125}I-ligand bound in the presence of 5 mg/ml BSA (Sigma), which gave identical values. The former method confirmed further that iodination did not significantly affect the virus-binding function of either FX or sgC. The amount of Ca^{2+}-independent binding was measured as above with 5 mM EDTA being included in the equilibrium mixture and sucrose cushion. The effect of 1 mg/ml low-molecular-mass heparin (Sigma) on FX and sgC binding to HSV1 was also studied.

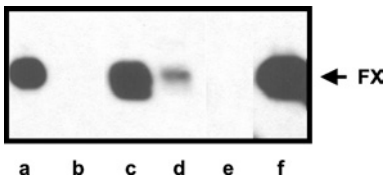

Figure 1 FX associates with the NS strain

Initial binding of FX to the NS strain was determined by Western blot analysis. Following a 35 min incubation period, the amount of FX in HBS/PEG/Ca^{2+}/benzamidine before centrifugation (a) or after in the mock-treated pellet (b) was determined. The NS strain was also added to the FX mixture and incubated as above with the amount of FX being assessed before (c) or after (d) centrifugation. Purified HSV1 (e) or purified FX (f) (0.1 μM) were also examined.

Western blotting

To confirm and quantify the relative amount of gC and TF on purified HSV1 strains, virus proteins were subjected to SDS/12 % PAGE followed by electrotransfer on to PVDF membranes and evaluated by Western blot analysis [23]. Antigen was detected by incubation with 1.2 μg/ml anti-gC mAb (monoclonal antibody) [25] or 1.0 μg/ml anti-TF mAb (American Diagnostica, product 4503). To visualize bands, a secondary horseradish-peroxidase-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) and enhanced chemiluminescent substrate (ECL Plus, Pierce) were used. As shown previously, TF was associated with all virus preparations used, while gC was present in the NS and rns strains only.

In preliminary experiments to determine whether FX bound to HSV1, Western blot analysis was carried out on the virus pellet following ultracentrifugation. The appearance of FX with the HSV1 or mock-treated pellet was determined by incubating the electrotransferred proteins with 1 μg/ml anti-FX polyclonal antibody (Assera X) followed by visualization as above.

Data analysis

All data points are means $\pm$ S.D. for triplicate experiments. The data were fitted using GraphPad software to a simple one-site binding model using non-linear regression and the equation [ligand bound] $= (B_{max}[\text{total ligand}])/(K_d + [\text{total ligand}])$, where B_{max} is the maximum number of binding sites and K_d is the apparent average dissociation constant. Although multiple types of binding are hypothesized, the simplest equation to describe binding was used to derive total B_{max} (ligand molecules bound/vp) and average K_d (μM), because individual binding site parameters could not be derived accurately using higher-order equations to fit these data.

RESULTS

FX binding to HSV1 in the presence of Ca^{2+}

To show direct FX binding to purified HSV1, differential sedimentation was used to separate bound from free ligand. The use of three related virus strains, NS, ns-1 and rns, enabled evaluation of the contribution of gC. As a preliminary study, the amount of FX remaining associated with the NS strain was followed by Western blot analysis (Figure 1). This experiment revealed detectable FX in the pellet only in the presence of HSV1, indicating binding. Furthermore, these data confirmed that the FX used was not proteolytically changed during the time course of the experiment, which could result in altered binding.

While qualitatively informative, the antigenic detection of FX bound to the virus pellet was not sensitive enough to quantify the

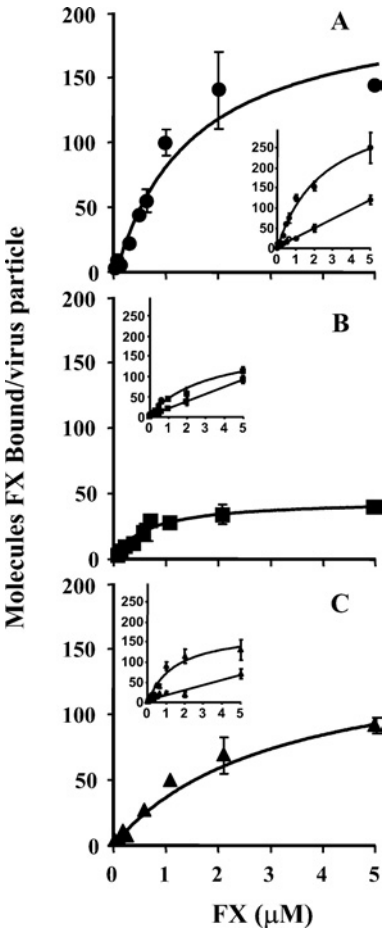

Figure 2 FX binds to HSV1 and is partially dependent on the presence of gC

Increasing amounts of ^{125}I-FX were incubated with NS (●), ns-1 (■) or rns (▲) strains in HBS/PEG/Ca^{2+}/benzamidine for 35 min at 21 °C. The reaction mixture was then layered on to the same buffer containing 10 % sucrose and centrifuged at 13000 ***g*** for 10 min, and the amount of radioactivity associated with the virus pellet was determined. The amount of specific binding illustrated was determined by subtracting from the total amount bound (insets, upper line) the amount of activity generated in the presence of a 40-fold excess of unlabelled ligand (insets, lower line) or BSA, which gave identical results. The binding data were fitted using an equation for a single type of binding.

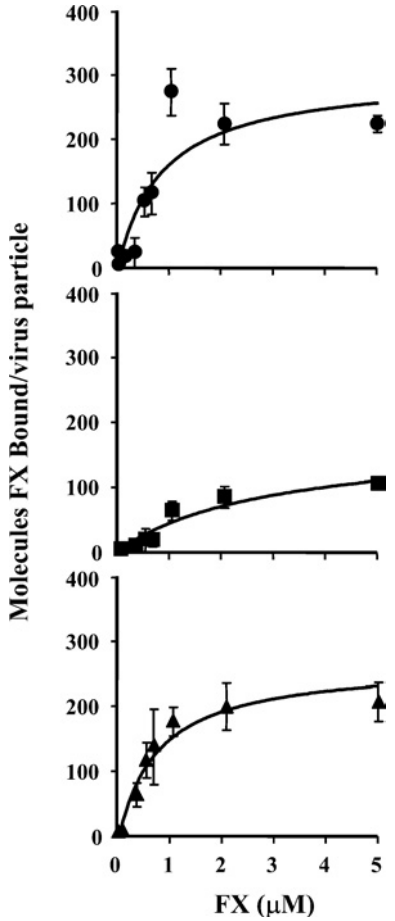

Figure 3 Soluble gC increases the binding of FX to HSV1

Various amounts of ^{125}I-FX were incubated with NS (●), ns-1 (■) or rns (▲) strains in HBS/PEG/Ca^{2+}/benzamidine plus 0.5 μM sgC for 35 min at 21 °C. Following incubation, the bound and free ligand were separated, and specific binding was calculated and fitted as in Figure 2.

interaction. Therefore radioiodinated FX was used as a detection method. The data clearly demonstrated a saturable FX–HSV1 interaction (Figure 2). ^{125}I-FX was displaced by unlabelled FX (Figure 2, inset) indicating that the iodination procedure was not responsible for binding and was consistent with only a marginal change in FX clotting function that we observed after iodination (results not shown). The B_{max} derived for FX on the NS strain was 206 ± 24 (Figure 2A) and was reduced to approx. 20 % for the ns-1 strain, 43 ± 5 (Figure 2B). Illustrating the importance of gC in FX binding to the virus, the rns strain was able to bind 136 ± 24 molecules of FX (Figure 2C). Demonstrating further an effect of gC on FX–virus binding, the ns-1 strain had a K_d of 0.69 ± 0.20 for FX binding, which was significantly lower than for both the NS (1.51 ± 0.39) and rns (3.13 ± 1.03) strains, and indicates that the gC-mediated FX interaction is weaker than the other contributing FX interactions on the virus surface in the presence of Ca^{2+}.

Effects of sgC on FX binding to HSV1 in the presence of Ca^{2+}

The demonstration that the NS strain binds nearly five times as much FX at saturation compared with the ns-1 strain implies that gC is part of a viral receptor for FX. To elaborate upon this observation, a constant amount of sgC was included in the ^{125}I-FX–virus equilibrium assay. The enzymatic studies published previously suggested that the half-maximal enhancement of FX activation by sgC in the presence of virus and FVIIa was approx. 0.5 μM [23]. We therefore measured the effect of this concentration of sgC on ^{125}I-FX binding in the present study. To further ensure consistency with the earlier work, the purified sgC preparations used in the present study had similar FXa-generating activity in the presence of FVIIa (results not shown). The addition of sgC to the ^{125}I-FX–virus equilibrium reaction mixture (Figure 3) was observed to significantly increase the maximum number of ^{125}I-FX binding sites on all three virus strains by approx. 100 molecules/vp.

sgC binding to HSV1 in the presence of Ca^{2+}

One explanation to account for our finding that sgC enhances FX–HSV1 binding is that sgC itself interacts directly with the virus. To demonstrate the possible interaction, sgC was radioiodinated, and binding to the individual virus strains was determined as for FX. The results confirmed that a direct sgC–HSV1 interaction exists. Each of the HSV1 strains tested was shown to bind approx. 100 molecules of sgC per vp: NS, 121 ± 6; ns-1, 126 ± 13; rns, 92 ± 13 (Figure 4). The K_ds for sgC binding to each virus were also

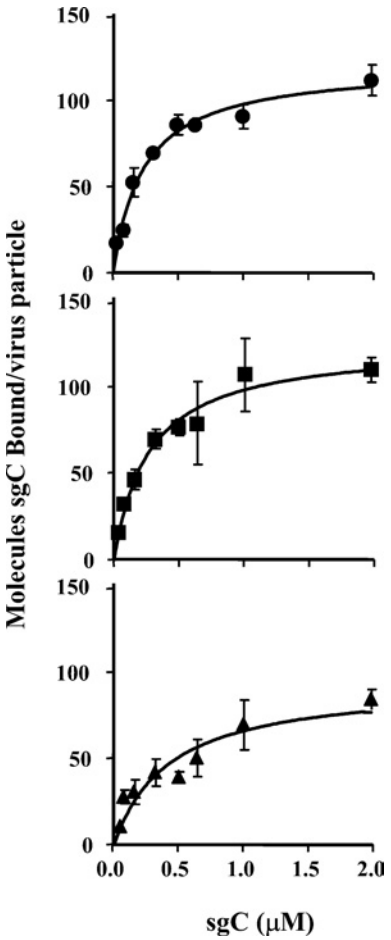

Figure 4 Soluble gC binds to HSV1

Increasing concentrations of ^{125}I-sgC were incubated with NS (●), ns-1 (■) or rns (▲) strains in HBS/PEG/Ca^{2+}/benzamidine for 35 min at 21 °C. Following incubation, the bound and free ligand were separated, and specific binding was calculated and fitted as in Figure 2.

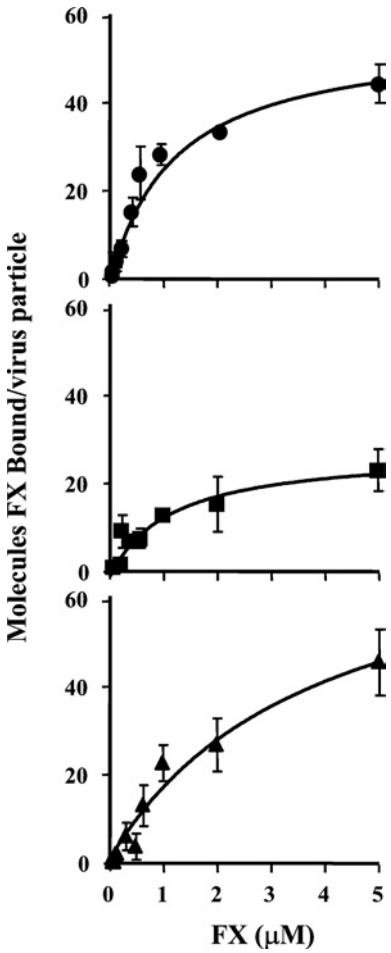

Figure 5 FX binding to HSV1 is decreased in the absence of Ca^{2+}

^{125}I-FX was titrated in the presence of NS (●), ns-1 (■) or rns (▲) strains in HBS/PEG/Ca^{2+}/benzamidine plus EDTA and was incubated for 35 min at 21 °C. Following incubation, the bound and free ligand were separated, and specific binding was calculated and fitted as in Figure 2.

comparable (NS, 0.23 ± 0.04; ns-1, 0.27 ± 0.09; rns, 0.44 ± 0.16), indicating that gC is not involved in sgC binding to HSV1.

FX binding to HSV1 in the absence of Ca^{2+}

Ca^{2+} is known to facilitate the association of FX with aPL [29], which is accessible on the HSV1 surface [22]. In order to eliminate this Ca^{2+}-dependent effect of FX binding, EDTA was added to chelate bivalent cations, and the effects on the HSV1–FX interaction were studied. All three virus strains were shown to bind ^{125}I-FX in the absence of Ca^{2+}, demonstrating Ca^{2+}-independent binding and also suggesting aPL-independent sites for FX on the virus (Figure 5). The NS and rns strains bound approx. 2-fold more FX (50 molecules/vp) than the ns-1 strain (25 molecules/vp), demonstrating that gC is partially involved in Ca^{2+}-independent FX binding to the virus surface. Although the very low amount of binding made it difficult to unambiguously derive a K_d in the absence of Ca^{2+}, there were insignificant differences between all three virus strains.

Effects of sgC on FX binding to HSV1 in the absence of Ca^{2+}

To characterize further the effect of sgC on the HSV1–FX interaction, Ca^{2+} was chelated by the addition of EDTA to the reaction mixture, and the effects on ^{125}I-FX-binding were quantified. In contrast with our findings in the presence of Ca^{2+}, 0.5 μM sgC was shown to have an inhibitory effect on FX binding to HSV1 in the absence of Ca^{2+} (Figure 6). This implies that sgC-dependent FX binding to the virus requires Ca^{2+}. The number of FX-binding sites to all three virus strains was equally reduced 2-fold, with no obvious effects on K_d, indicating that viral gC did not play a role in the observed inhibition.

sgC binding to HSV1 in the absence of Ca^{2+}

One possible explanation for the sgC-mediated FX binding to HSV1 is that sgC–virus interactions are Ca^{2+}-dependent. To test this hypothesis, Ca^{2+} was chelated by EDTA, and ^{125}I-sgC binding to HSV1 was measured. The results demonstrated that ^{125}I-sgC binding was nearly eliminated (fewer than eight molecules/vp) in the absence of Ca^{2+} for all three strains (Figure 7), indicating a strong dependence on Ca^{2+}.

Effect of heparin on FX binding to HSV1

To investigate the involvement of the known heparin-binding sites on FX [30] and gC [31] in the FX–gC interaction, the association of ^{125}I-FX with HSV1 was measured in the presence of soluble low-molecular-mass heparin. Including heparin was found to significantly inhibit the amount of FX binding to NS and rns strains, but had no discernable effect on the amount of FX binding

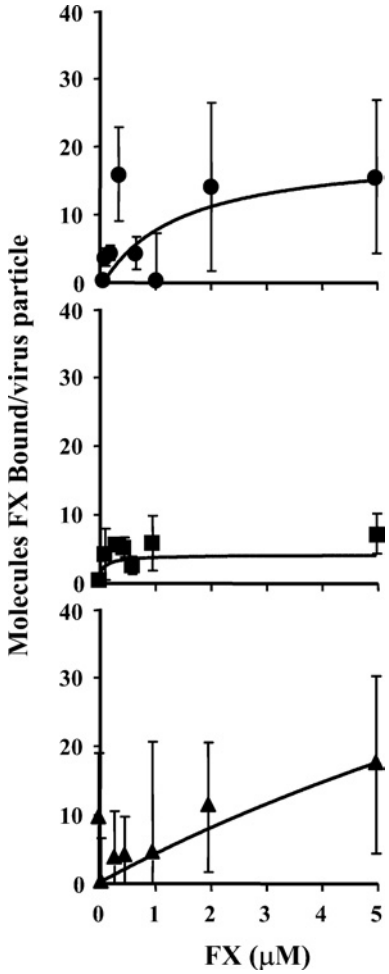

Figure 6 sgC-dependent binding of FX to HSV-1 is reduced in the absence of Ca^{2+}

^{125}I-labelled FX was incubated with NS (●), ns-1 (■) or rns (▲) strains in HBS/PEG/Ca^{2+}/benzamidine plus 0.5 μM sgC and EDTA for 35 min at 21 °C. Following incubation, the bound and free ligand were separated, and specific binding was calculated and fitted as in Figure 2.

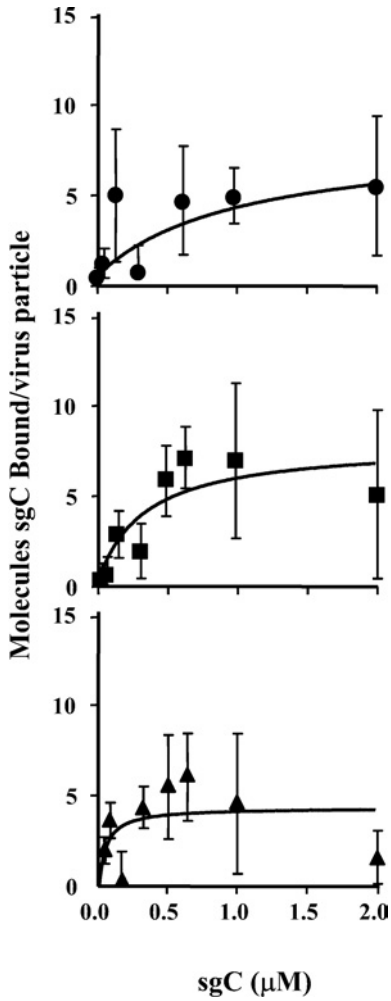

Figure 7 sgC binding to HSV1 is dependent on the presence of Ca^{2+}

Increasing concentrations of ^{125}I-sgC were incubated with NS (●), ns-1 (■) or rns (▲) strains in HBS/PEG/Ca^{2+}/benzamidine plus EDTA for 35 min at 21 °C. Following incubation, the bound and free ligand were separated, and specific binding was calculated and fitted as in Figure 2.

to the ns-1 strain (Figure 8). In contrast, heparin had no effect on ^{125}I-sgC binding to all three HSV1 strains (results not shown). These data suggested that the FX–gC complex is near to or involves the heparin-binding site on at least one of the binding partners and provides further evidence for a direct gC–FX interaction.

DISCUSSION

Our laboratory group reported previously that virus-encoded gC and the solubilized recombinant form, sgC, accelerate FX activation by FVIIa on the surface of HSV1 [23]. An FX interaction with the virus and contributions of gC or purified sgC to FX binding were implied by these studies. Previous cross-linking experiments indicated further that gC expressed on the surface of HSV1-infected endothelial cells is within 10 Å (1 Å = 0.1 nm) of cell-bound FX [17]. To provide direct evidence, ^{125}I-labelled FX was used in the present work to quantify the FX–virus association. As hypothesized, we now report that FX binds directly to HSV1 (summarized in Table 1). The interaction with the NS strain was mediated by a K_d of 1.5 μM with 206 sites/vp in the presence of Ca^{2+}. FX binding to the ns-1 strain was observed to have a reduced number of binding sites (43 sites/vp) and higher affinity ($K_d = 0.7$ μM), demonstrating a direct contribution of gC to overall binding. Our current data show that the virus surface itself has more than one distinct type of FX–HSV1 interaction and that the preponderance of FX binding involves gC. Thus the association between FX and gC is likely to be an important component of the viral FX-activating complex that enables HSV1 to bypass the normal cellular requirement for initiating coagulation.

To support further the contribution of gC in FX–HSV1 interactions, addition of sgC (0.5 μM) to the ns-1 strain increased both the number of FX-binding sites and the K_d, shifting the overall binding parameters toward those of the NS and rns strains. One explanation accounting for the contribution of sgC to FX–virus binding is that sgC itself also interacts directly with the virus to provide FX receptors. Addressing this possibility, ^{125}I-sgC binding was measured, and, at saturating concentrations, approx. 100 molecules bound to all three virus strains ($K_d = 0.3$ μM). Consistent with a 1:1 interaction between sgC and FX on the virus surface, 100 FX-binding sites were added in the presence of sgC. Since the ns-1 and rns strains interacted comparably with sgC, a virus constituent other than gC is suggested to have a role in sgC binding. This as yet unknown binding partner may be responsible for the enhanced contribution of sgC in FX activation by FVIIa found when purified HSV1 was present [23].

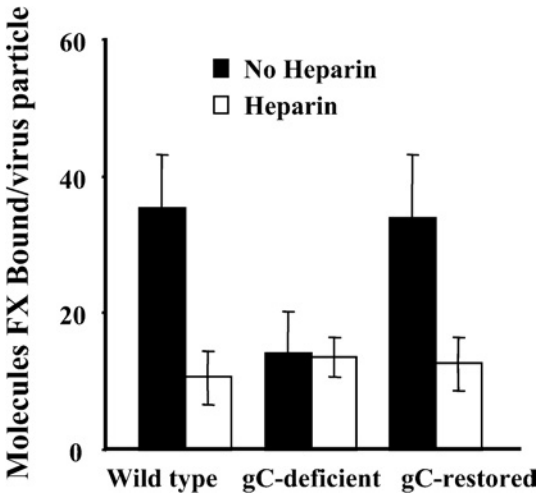

Figure 8 Heparin reduces the amount of FX binding to gC-competent HSV1 strains

^{125}I-FX (0.5 μM) was incubated with NS (Wild type), ns-1 (gc-deficient) or rns (gC-restored) strains in HBS/PEG/Ca^{2+}/benzamidine plus low-molecular-mass heparin for 35 min at 21 °C. Following incubation, the bound and free ligands were separated, and specific binding was calculated as in Figure 2.

Table 1 Summary of FX–virus binding parameters

		Ca^{2+}		No Ca^{2+}	
Virus	Ligand	K_d	B_{max}	K_d	B_{max}
NS	^{125}I-FX	1.51 ± 0.39	206 ± 24	1.23 ± 0.27	54 ± 5
	^{125}I-FX + sgC	0.85 ± 0.33	299 ± 45	1.42 ± 1.99	20 ± 12
	^{125}I-sgC	0.23 ± 0.04	121 ± 6	0.98 ± 1.77	8 ± 7
ns-1	^{125}I-FX	0.69 ± 0.20	43 ± 5	1.14 ± 0.43	29 ± 5
	^{125}I-FX + sgC	2.00 ± 1.24	150 ± 33	0.09 ± 0.14	5 ± 2
	^{125}I-sgC	0.27 ± 0.09	126 ± 13	0.32 ± 0.37	8 ± 3
rns	^{125}I-FX	3.13 ± 1.03	136 ± 24	3.78 ± 1.54	83 ± 19
	^{125}I-FX + sgC	0.76 ± 0.27	261 ± 38	19 ± 142	87 ± 516
	^{125}I-sgC	0.44 ± 0.16	92 ± 13	0.05 ± 0.08	4 ± 1

In contrast with the enhancing effect of sgC on FX binding to HSV1 in the presence of Ca^{2+}, sgC caused inhibition when Ca^{2+} was chelated. A definitive explanation is not yet available. However, our observation that the sgC–HSV1 interaction requires Ca^{2+} may suggest that a Ca^{2+}-independent association between sgC and FX in solution prevents FX from interacting with Ca^{2+}-independent virus sites. Thus Ca^{2+}-dependent binding of sgC to the virus may facilitate sgC-meditated FX–HSV1 interactions.

Earlier studies demonstrated the presence of accessible aPL on the surface of HSV1 [22], which probably contributes to the observed FX-binding shown in the present study in the presence of Ca^{2+}. However, the reported K_d of approx. 0.3 μM for FX binding to synthetic phospholipid vesicles [32] is considerably lower than we observed for the NS strain ($K_d = 1.5$ μM). This difference suggests that aPL within the HSV1 envelope is not the primary basis for FX association with HSV1. aPL-mediated interactions may play a greater role in FX binding to the ns-1 strain, as indicated by the lower observed K_d (0.7 μM). Measurable association of FX with all three HSV1 strains in the absence of Ca^{2+} confirms that there are aPL-independent mechanisms. We therefore propose at least three distinct types of FX binding to HSV1: (i) gC-mediated, (ii) aPL-mediated, and (iii) aPL- and gC-independent (possibly TF), although the latter represents less than 15 % of binding sites.

From the previous kinetic studies conducted at approximately physiological concentrations of FX (100 nM) and FVIIa (5 nM), a significant amount of FXa was shown to be generated on the surface of HSV1 due in part to virus-associated TF [22,23]. An approximate K_m estimated from these studies is below 100 nM. Purified TF–FVIIa has been shown previously to have a high affinity for FX, also inferred from kinetic studies [20]. To our knowledge, there have been no reports on the direct interaction between TF and FX, presumably due to a prohibitively weak affinity in the absence of FVIIa. In the present study, FX binding was followed without the contribution of FVIIa, to derive intrinsic binding parameters and prevent potential effects on the FX–virus equilibrium due to activation. The K_d values we derived were consequently above the physiological concentration of FX, as predicted. We anticipate that FVIIa will play an important role to enhance the interaction between FX and HSV1.

Both FX and gC have well-documented heparin-binding properties that affect function [31,33]. To begin to understand regions in FX or gC that may participate in the interaction, the effects of low-molecular-mass heparin (< 5 kDa) on FX binding to HSV1 was determined. We found that the addition of heparin significantly decreased the amount of FX associated with the virus, but only for the HSV1 strains containing gC. These data indicate that the heparin-binding regions on FX and/or gC may be close to or participate directly in the FX–gC interaction.

The ability of HSV1 to bind and subsequently activate FX may provide a biochemical explanation for clinical observations that link the virus with vascular disease [2–4]. Although the present work focused on HSV1, other members of the Herpesvirus family, including HSV2 (herpes simplex virus type 2) and CMV (cytomegalovirus) have been shown to possess TF and aPL, enabling tenase assembly and FXa generation. Correcting for known differences in virus surface area [34], the K_d and number of sites per virus observed for the ns-1 strain is similar to previously reported FX binding to CMV [35]. Outside the Herpesvirus family, HIV type 1 and 2 are both known to contain a higher abundance of aPL than the host cell membrane [36], while host cells infected with measles virus [37] or murine hepatitis virus [38] display increased thrombogenesis. Thus the observations of the present study that imply the evolution of the virus to activate the host coagulation pathway may suggest a more general contribution of recurrent virus infection to the risk of vascular disease.

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